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Do limited cold tolerance and shallow depth of roots contribute to yellow-cedar decline?

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A B S T R A C T

It has been proposed that yellow-cedar (*Callitropsis nootkatensis*) decline is initiated by the freezing injury of roots when soils freeze during times of limited snowpack. To explain the unique susceptibility of yellow-cedar in contrast to co-occurring species, yellow-cedar roots would need to be less cold tolerant and/or more concentrated in upper soil horizons that are prone to freezing. We measured the root cold tolerance and used concentrations of foliar cations as an assay of rooting depth for five species in one forest in Ketchikan, Alaska. Species evaluated were yellow-cedar, western redcedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*), mountain hemlock (*Tsuga mertensiana*), and Sitka spruce (*Picea sitchensis*). Roots were collected in November 2007 and January, March and May 2008; foliage was collected in January 2008. Soil samples from surface and subsurface horizons were analyzed for available calcium (Ca) and aluminum (Al) to compare with foliar cation concentrations. Across all dates the sequence in hardiness from the least to most cold tolerant species was (1) yellow-cedar, (2) western redcedar, (3) western and mountain hemlock, and (4) Sitka spruce. Yellow-cedar and redcedar roots were less cold tolerant than roots of other species on all sample dates, and yellow-cedar roots were less cold tolerant than redcedar roots in January. Yellow-cedar roots were fully dehardened in March, whereas the roots of other species continued to deharden into May. Yellow-cedar roots exhibited the highest electrolyte leakage throughout the year, a pattern that suggests the species was continuously poised for physiological activity given suitable environmental conditions. Yellow-cedar and redcedar had higher foliar Ca and lower Al concentrations, and greater Ca:Al ratios than the other species. Yellow-cedar had higher foliar Ca and Ca:Al than redcedar. Soil measurements confirmed that the upper horizon contained more extractable Ca, less Al and higher Ca:Al than the lower horizon. Considering the distribution of Ca and Al in soils, we propose that concentrations of Ca and Al in yellow-cedar and redcedar foliage reflect a greater proportional rooting of these species in upper soil horizons compared to other species tested. Greater Ca and Ca:Al in the foliage of yellow-cedar suggests shallower rooting compared to redcedar, but broad similarities in foliar cation profiles for these species also highlight some overlap in rooting niche. Our data indicate that both limited root cold tolerance and shallow rooting likely contribute to the unique sensitivity of yellow-cedar to freezing injury and decline relative to sympatric conifers.

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1. Introduction

Yellow-cedar (*Callitropsis nootkatensis* (D. Don) Florin ex D.P. Little) is an ecologically, economically and culturally important tree species that has an extensive native range from the northern Klamath Mountains of California to Prince William Sound in Alaska (AK). The species is limited to high elevations throughout most of its range, except in northern regions where it grows from near timberline down to sea level (Harris, 1990). Yellow-cedar is a slow-growing tree that tolerates poor growing sites, and which diverts considerable resources toward protection from biotic stressors (Hennon and Shaw, 1997). Despite this protection, yellow-cedar has experienced dramatic mortality (Hennon and Shaw, 1997; Hennon et al., 2005) now estimated to extend over 200,000 hectares in AK (Lamb and Winton, 2010) and nearly 50,000 hectares in British Columbia (Westfall and Ebata, 2009). The extensive mortality of this species, referred to as yellow-cedar decline, is a classic example of forest declines, which are generally recognized as wide-spread, long-term, and having either complex or unresolved etiologies (Manion and Lachance, 1992). Consistent with the defensive niche of the species (e.g., being relatively free from insect damage with its wood remarkably durable to fungal attack; Harris, 1990), yellow-cedar decline is not believed to be associated with

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any biotic causal agent (Hennon and Shaw, 1997). Instead, it has been proposed that yellow-cedar decline results when climatic warming reduces the depth of winter snowpacks, and increases the likelihood of soil freezing and subsequent fine root injury when cold continental air periodically moves over the region in spring (Hennon et al., 2006). Predisposing factors such as wet soils that limit rooting depth and open canopies that increase exposure to ambient freeze–thaw cycles (Hennon et al., 2010) may combine with reductions in winter snow accumulation to enhance the likelihood and severity of soil freezing that can kill sensitive roots (Hennon et al., 2006, 2010; Schaberg et al., 2008). Canopy decline in spring would follow root mortality as seasonal increases in transpiration result in chronic foliar water shortages when damaged root systems cannot fully resupply leaves (Hennon et al., 2006; Schaberg et al., 2008). With this scenario, crown decline would be greatest and proceed more quickly when root mortality was high (Schaberg et al., 2008).

Field-based data are consistent with the hypothesis that reductions in winter snowpack contribute to yellow-cedar decline (Hennon et al., 2010). For example, Beier et al. (2008) documented a trend toward warmer mid-winter temperatures during the 1990s and less snow since the 1950s in the region experiencing decline. In addition, a comparison of aerial sketch maps of the spatial extent of yellow-cedar decline and geographically explicit snowpack estimates at both broad (regional) and mid (island) scales show that decline is limited to low- and mid-elevations – areas of low predicted snow accumulation (Hennon et al., 2006). At a finer spatial scale, vegetation, hydrologic, soil and temperature data from plots on 100 m grids in two small watersheds have helped to document that injury is associated with wet soils, decreased canopy cover, and greater exposure to temperature fluctuations and extremes (D'Amore and Hennon, 2006; Hennon et al., 2006, 2010). Importantly, data from these plots indicate that soils can reach damaging sub-freezing temperatures for sustained periods when soils are not protected by snow (Hennon et al., 2010). Beier et al. (2008) demonstrated that, despite a warming trend and reduced snow through the 1900s in southeast AK, the frequency and intensity of cold periods in late winter and early spring persisted. These cold periods that presumably cause injury to yellow-cedar occur when high pressure weather systems push cold air from adjacent interior British Columbia and the Yukon Territory over southeast AK.

Experimental data also support the possibility that a lack of snow cover increases soil freezing that injures roots and triggers whole-tree decline. In a soil protection experiment, yellow-cedar seedlings either had their root systems covered with simulated snow (perlite) during early winter or were left exposed to ambient air temperatures (Schaberg et al., 2008). The roots of seedlings from both treatments were cold tolerant to only about −5 °C (Schaberg et al., 2008). Unprotected soils reached temperatures well below −5 °C on at least eight occasions during the experiment, whereas soils in the simulated snow protection treatment remained at or near 0 °C throughout this period (Schaberg et al., 2008). Seedlings in the simulated snow treatment showed no root injury (measured as increased electrolyte leakage and visible injury), whereas seedlings without protection had significant root injury, that was followed by foliar decline and eventual mortality. The pattern of initial root injury followed by later foliar damage and mortality documented for unprotected seedlings (Schaberg et al., 2008) corresponded to field reports of the nature and sequence of injury that characterize yellow-cedar decline (Hennon et al., 1990).

Current spatial, temporal and mechanistic evidence are consistent with the hypothesis that low snowpack results in an increased risk of root freezing injury that instigates yellow-cedar decline. However, factors that cause yellow-cedar to be uniquely vulnerable to root freezing injury relative to other co-occurring species have not been identified. Limited root cold tolerance or a high proportion of roots in shallow soils could both increase yellow-cedar's vulnerability to root freezing injury. D'Amore et al. (2009) recently proposed that yellow-cedar and western redcedar (*Thuja plicata* (Donn ex D. Don)) may utilize a shallow rooting habit in organic soils to promote nitrogen (N) assimilation as nitrate (NO_3^-) and accumulate calcium (Ca^{+2}) as a counter-ion during uptake. Preferential rooting in shallow organic soils could benefit the cedars by providing access to a form of N (NO_3^-) that is less exploited by competing species (D'Amore et al., 2009). However, shallow rooting could also have a negative tradeoff – an increased risk of root freezing when soils were not protected from ambient temperature lows by an insulative snowpack. Indeed, using foliar calcium (Ca) concentrations as an indicator of root abundance in surface (Ca-rich) versus sub-surface (Ca-poor) soil horizons, D'Amore et al. (2009) speculated that yellow-cedar may concentrate more roots in shallow organic soil horizons than western redcedar – making yellow-cedar more vulnerable to root freezing injury. Despite this initial comparative analysis, to date no comprehensive assessment of the two factors most likely to make yellow-cedar uniquely vulnerable to root freezing injury relative to sympatric species (i.e., the cold tolerance and depth of roots) has been conducted.

To assess if limited cold hardiness and/or a shallow depth of rooting contribute to yellow-cedar's vulnerability to decline, we measured the seasonal (fall through spring) cold tolerance of roots, and used ratios of foliar cations as an assay of rooting depth for five coniferous species growing together near Ketchikan, AK. Species evaluated were two species from the family Cupressaceae: yellow-cedar (prone to decline) and western redcedar (thought to occupy a similar ecological niche as yellow-cedar), and three Pinaceae species with divergent ecologies that are not declining: western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), mountain hemlock (*T. mertensiana* (Bong.) Carr.), and Sitka spruce (*Picea sitchensis* (Bong. Carr.)).

2. Materials and methods

2.1. Site description and sampling

The research site was located in Ward Creek Valley 9.8 km northeast of Ketchikan, AK at 170 m elevation (Lat. 55.42853, Long. −131.68912). The site was located on the northwest side of Revillagigeddo Island, which is part of the Traitors Cove Metasediments geomorphological subsection (Nowacki et al., 2001). These geologic deposits have been heavily re-worked by glacial activity leading to rolling hills, lakes, and extensive glacial drift deposits. The forest chosen for study is located on a footslope above Ward Creek, the main drainage for the watershed. Soils range from poorly-drained Histosols, somewhat poorly drained Inceptisols and Spodosols, to moderately well-drained Spodosols depending on microsite characteristics. The forest is typical of palustrine forested wetlands (USFWS, 2009) with a diverse assemblage of tree species including yellow-cedar, western redcedar, Sitka spruce, western hemlock, and mountain hemlock.

Trees that appeared healthy and >20 cm diameter at DBH (1.3 m) were selected along a transect that was located within the forested wetland where all five tree species were growing. Six groups of trees that included one from each of the five species (a total of 30 trees) were randomly chosen for study from five locations along this transect. From each tree, fine roots (<2 mm in diameter) were collected from a randomly selected region of soil around each tree on four sampling dates: 5 November, 2007; 14 January, 10 March, and 5 May, 2008. Soil regions were selected using a directional (azimuth) bearing and were sampled without replacement. Excavations began at the bole of trees to ensure that collected roots belonged to the sample tree, and continued along coarse roots until a sufficient quantity of fine roots could be collected. Fine roots were carefully separated from soil by hand, wrapped in damp paper towels, placed in plastic bags, and stored in a cooler in the field. Sun-lit foliage was collected from each tree using a pole pruner on 14 January, 2008 – a time when root cation uptake is typically minimal and foliar cation concentrations are stable (DeHayes et al., 1997). Immediately following field collection, root and foliar tissues were shipped overnight to the University of Vermont for assessments of cold tolerance and membrane integrity (roots) or cation concentrations (foliage).

2.2. Temperature monitoring

Air temperature loggers (Hobo water temperature pro v2, Onset Computer Corp., Bourne, MA) and soil temperature loggers (Tidbit v2, Onset Corp.) were placed at four locations along the sampling transect in November 2007. One air temperature logger was placed on the north side of a tree 2 m above the soil surface, and two soil temperature loggers were buried 7.5 cm and 15 cm deep in the soil at each of the four locations. All loggers recorded temperature hourly (non-averaging) from November 2007 to May 2008. Daily minimum and maximum temperature values from the Ketchikan weather station (#504590, Western Regional Climate Center) located 8.3 km from the study site were used to display temperature trends during fall 2007 before temperature loggers were installed.

2.3. Cold tolerance

Cold tolerance was assessed by measuring the electrolyte leakage of fine root tissues following controlled exposure to a series of progressively lower test temperatures (Schaberg et al., 2008). Prior to cold tolerance assessments, roots were washed in cold tap water to remove soil and only light-colored roots that showed no visible damage were assessed for cold tolerance. Very few damaged roots were identified for any species over all sample periods. Following initial preparations, root tissues were rinsed in a Triton X-100 (wetting agent) – distilled water solution, then washed in a series of chilled distilled water baths to remove surface ions before being cut into 5-mm lengths to produce one bulked sample per tree. Subsamples of bulked tissue were measured volumetrically (approximately 0.3 ml or 0.1–0.2 g) and placed into 64-cell styrene trays for freezing tests in which each sample tray was exposed to a different test temperature. Freezing stress was imposed using the methods of Schaberg et al. (2008). Test temperatures were 4, 0, −2, −4, −6, −9, −12, −15, −18, −21, −24, −27, −30, −34 and −44 °C. The rate of freezing was $-6\,^{\circ}\mathrm{C\,h^{-1}}$ for all tests. Freezer temperature was held at least 20 min at each test temperature, after which one replicate tray was removed from the freezer, placed in a pre-cooled styrene foam container, and transferred to a separate freezer at −5 °C (except for test temperatures above −6 °C for which trays were transferred to a 4 °C refrigerator). For samples in the freezer, after trays equilibrated to −5 °C, they were transferred in foam containers to a refrigerator at 4 °C until thawed. A water solution with a wetting agent (3.5 mL of 0.1% v/v Triton X-100 – deionized water) at 4 °C was added to each cell, and sample trays were held in a high humidity cabinet and shaken at room temperature for 8 h. Initial conductivity of the effusate was measured using a multielectrode instrument (Wavefront Technology, Ann Arbor, MI), then samples were dried for at least 48 h at 50 °C to kill the tissue, soaked in fresh wetting agent solution for 24 h, and then the final conductivity was measured. Relative electrolyte leakage (REL), a measure of cell injury calculated as the proportion of initial to final conductivity, was used to estimate tissue cold tolerance measured as T_m, an estimate of LT_{50} (temperature at 50% cell mortality) (Strimbeck et al., 2008). T_m values were calculated for each tree via non-linear curve-fitting (JMP, SAS Institute, Cary, NC) using the following equation (Anderson et al., 1988):

$$Y_T = Y_{\min} + \frac{Y_{\max} - Y_{\min}}{1 + e^{k(T_m - T)}}$$

where Y_T is the REL value at temperature T, $Y_{\min}$ is the asymptotic REL value in uninjured tissue, $Y_{\max}$ is the asymptotic REL value at maximum freezing stress, k describes the steepness of the REL response to freezing stress, T is the temperature in °C, and T_m is the midpoint value of the REL sigmoid curve.

2.4. Membrane electrolyte leakage

Relative electrolyte leakage from plant cells is routinely used as a measure of membrane stability, and has been employed to detect changes in membrane integrity associated with inherent differences in cell viability (van Bilsen and Hoekstra, 1993) as well as damage from a range of imposed stresses such as dehydration (Kuhns et al., 1993), freezing (Schaberg et al., 2008), high temperatures (Ruter, 1996), osmotic stress (Zwiazek and Blake, 1991), and changes in mineral nutrition (Branquinho et al., 1997). Measurements of REL provide a sensitive indicator of tissue damage that is often detectable prior to the expression of visible injury (Schaberg et al., 2008). We used root REL data from tissues not exposed to experimental freezing stress (i.e., $Y_{\min}$ – the calculated baseline for the sigmoid curve fit to REL data that is used to calculate cold tolerance) to estimate baseline membrane integrity and test for incipient field injury for each tree. As per sampling criteria for cold tolerance assessments, visibly injured tissues were excluded when roots were sampled for REL measurements. Thus, REL data provide an estimate of damage to visibly uninjured tissues.

2.5. Foliar Ca and Al analyses

Cation analysis was assessed on the current-year foliage of hemlocks and spruce and the distal segments of primary shoots (the region assessed for physiologic comparison to current-year foliage) for cedars (Schaberg et al., 2005). Foliage was dried for two weeks at 65 °C, then samples were ground to pass a 2-mm sieve and digested by heating with nitric acid and hydrogen peroxide using a block digester (adapted from Jones and Case, 1990). Samples were analyzed for total foliar Ca and Al by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Perkin-Elmer Optima DV 3000, Perkin–Elmer Corp., Norwalk, CT). Eastern white pine needles from the National Bureau of Standards and Technology (SRM 1575a), sample duplicates, and blanks were analyzed for procedural verification. Assayed tissue standards were within 5% of certified values.

2.6. Soil Ca and Al analyses

The availability of Ca and Al in surface (0–5 cm) and sub-surface (15–20 cm depth) soil horizons was measured to compare with the foliar concentrations of these cations and assess their use as indicators of rooting depth. Soil samples were taken from the drip zone around three trees from each species ($n = 15$ trees total), which were selected by a stratified random approach to distribute the sampling locations across the site. Three zones around the tree were selected for sampling along azimuths (0°, 120°, and 240°) located 2 m from the tree bole. At each location a large block of soil was excavated and samples from the surface and subsurface were taken from the large excavated sample. Two samples from each block and depth were composited for chemical analysis (i.e., 3 samples × 2 depths for each tree). Duplicate samples were taken for bulk density calculation at each location around the tree by obtaining a volumetric sample of soil either by cutting a block of soil from organic material, or use of a volume sampler for mineral soil. The sampling was conducted as a depth-based approach, and all surface samples were organic soil material, while subsurface samples contained both organic and mineral material. Cation extractions were conducted according to methods described in Robertson et al. (1999). In the laboratory, 15 g of homogenized soil were mixed with 100 mL of 1.0 M KCl, shaken for 1 min, rested overnight, re-shaken for 1 min, rested for 45 min. A sample of the supernatant was taken and filtered and distributed in triplicate 20 mL subsamples. Extracted subsamples were filtered and analyzed using ICP-AES to quantify concentrations of extractable Ca and Al (mg nutrient kg^{-1} soil). Approximately 10% of samples were run in duplicate to ensure analytical accuracy. Bulk density samples were transferred to pre-weighed tins, weighed wet, and then dried to a constant weight at 65 °C. Bulk density values were calculated from the volume of sample and dry weight of the soil material.

2.7. Statistical analyses

Repeated measures ANOVAs were used to test for differences in T_{m} and REL attributable to month, species, and month × species. T_{m} values were adjusted using a Box-Cox transformation to satisfy the assumption of homogeneity of variances (Montgomery, 2001). To evaluate differences among species within individual months, 12 orthogonal contrasts were made by partitioning the degrees of freedom for the interaction term "month × species". For each of the 4 months, three contrasts were conducted for the five species evaluated (yellow-cedar:YC, western redcedar:RC, mountain hemlock:MH, western hemlock:WH, and Sitka spruce:SS): (1) YC and RC versus MH, WH, and SS, (2) YC versus RC, and (3) MH and WH versus SS ($4 \times 3 = 12$ contrasts). For the main factor "species" that had four degrees of freedom, we added a fourth contrast (WH versus MH). These *a priori* contrasts maximized statistical power for evaluating differences among species or groups of species hypothesized to have different ecological niches: (1) Cupressaceae versus Pinaceae, (2) yellow-cedar versus western redcedar, (3) hemlocks versus spruce, and when possible (4) western versus mountain hemlock. Specific differences in T_{m} and REL for the factor "month" were tested using the Tukey HSD test. Foliar cation data were also tested using the four orthogonal contrasts described above, and soil cation data were analyzed by depth. Regression analysis was used to assess the linear relationship between concentrations of Ca and Al in soil horizons and foliage. Differences were considered statistically significant if $P \leqslant 0.05$.

3. Results and discussion

3.1. Root cold tolerance

For all dates combined, the sequence in overall hardiness from the least to most cold tolerant species was (1) yellow-cedar, (2) western redcedar, (3) western and mountain hemlock, and (4) Sitka spruce (Fig. 1). Roots of all species followed the expected seasonal pattern – they increased in hardiness from November to January, decreased in hardiness from January to March, and lost additional cold tolerance from March to May (Fig. 1). However, there was a significant month × species interaction for root cold tolerance ($P < 0.01$), which was driven by the comparatively minor changes in cold tolerance for some species (most notably yellow-cedar) versus the more typical seasonal fluxes in cold tolerance for other species (e.g., Sitka spruce) (Fig. 2). Yellow-cedar developed minimal winter hardiness, and appeared fully dehardened by March (Fig. 2). The range in monthly cold tolerance means over the course of the experiment was 1.5 °C for yellow-cedar, 3.4 °C for western redcedar, 3.4 °C for mountain hemlock, 4.3 °C for western hemlock, and 5.3 °C for Sitka spruce. Orthogonal contrasts for the month × species interaction indicate that (1) yellow-cedar and western redcedar were less cold tolerant than all other species on all dates, (2) yellow-cedar was less cold tolerant than western redcedar in January, and (3) the hemlocks were less cold tolerant than Sitka spruce in January and March (Fig. 2).

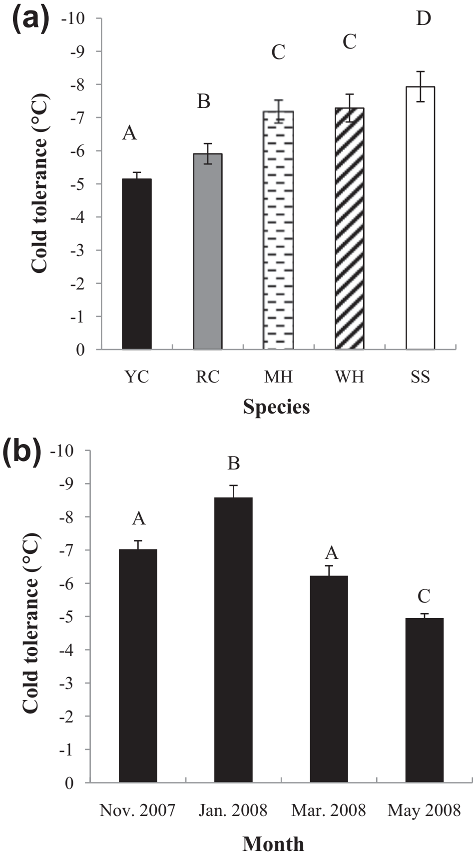

Fig. 1. (a) Differences in overall mean (±SE) cold tolerance (T_{m}) of fine roots for five species: yellow-cedar (YC), western redcedar (RC), mountain hemlock (MH), western hemlock (WH), and Sitka spruce (SS) growing in Ketchikan, Alaska during the 2007–2008 cold season. Species means with different letters are significantly different ($P \leqslant 0.05$) based on the following orthogonal contrasts: (1) YC and RC versus MH, WH, and SS, (2) YC versus RC, (3) MH and WH versus SS, and (4) MH versus WH. (b) Differences in mean (±SE) cold tolerance (T_{m}) of fine roots of all species for each sample month. Monthly means with different letters are significantly different ($P \leqslant 0.05$) based on Tukey HSD test.

Maximum hardiness levels in January were −6.2 (±0.4) °C for yellow-cedar, −7.8 (±0.5) °C for western redcedar, −8.8 (±0.6) °C for mountain hemlock, −9.3 (±0.7) °C for western hemlock, and −10.6 (±0.5) °C for Sitka spruce. Mid-winter cold tolerance estimates for yellow-cedar roots reported here were within 1 °C of maximum levels previously reported for the roots of yellow-cedar seedlings (Schaberg et al., 2008), and estimates for the other species were close to the −8 to −12 °C range in midwinter cold tolerance averages reported for the roots of other temperate conifers (e.g., Coleman et al., 1992; Bigras and Calmé, 1994). By comparison, boreal conifers consistently exhibit greater levels of root cold tolerance (e.g., −20 °C for Scots pine (*Pinus sylvestris* L.) and −25 °C for Norway spruce (*Picea abies* (L.) Karst.); Lindström and Stattin, 1994).

Yellow-cedar was less cold tolerant than western redcedar in mid-winter – a time when air temperatures were lowest and

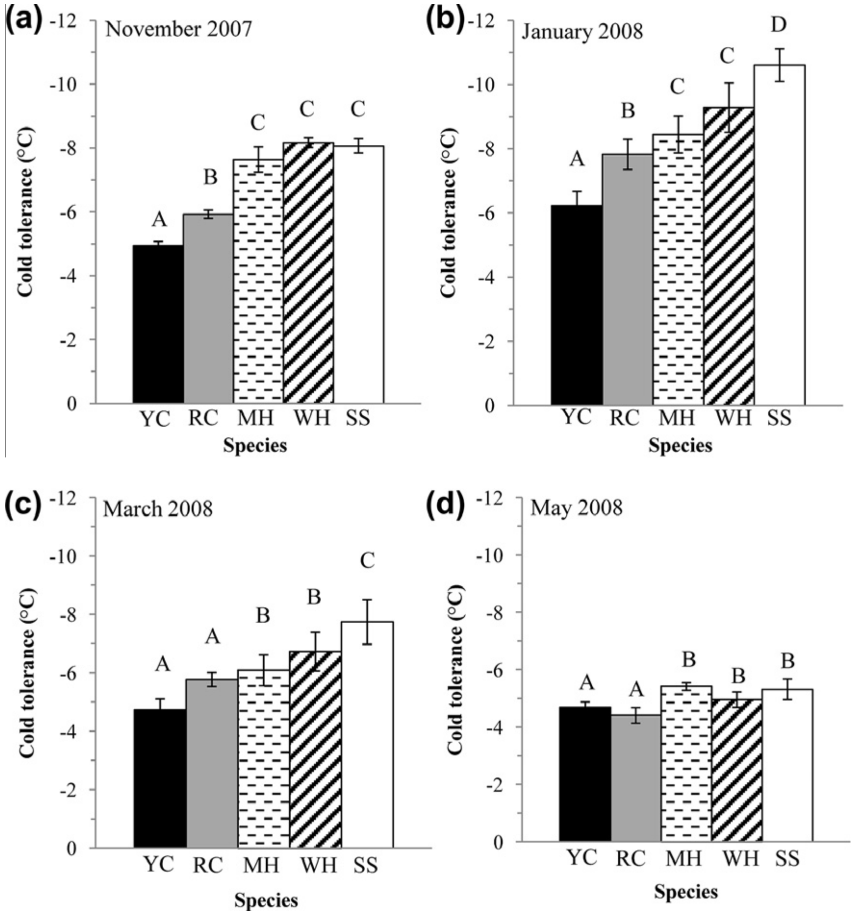

Fig. 2. Differences in mean (±SE) cold tolerance (T_m) of fine roots of yellow-cedar (YC), western redcedar (RC), mountain hemlock (MH), western hemlock (WH) and Sitka spruce (SS) growing together in Ketchikan, Alaska and assessed on four dates: (a) November 2007, (b) January 2008, (c) March 2008, and (d) May 2008. Per sampling date, treatment means with different letters are significantly different ($P \leqslant 0.05$) based on the following orthogonal contrasts: (1) YC and RC versus MH, WH, and SS, (2) YC versus RC, and (3) MH and WH versus SS.

before consistent spring warming occurred (Fig. 3). Although yellow-cedar appeared more vulnerable to freezing injury in January, mean differences in cold tolerance between the cedars were modest (1.6 °C). It is possible that this small difference is consequential because these thresholds in freezing tolerance are so close to actual temperature lows reached in soils in southeast AK when snow cover is lacking (e.g., −5 °C and lower; D'Amore and Hennon, 2006; Hennon et al., 2010). However, it is also possible that factors other than differences in cold tolerance (e.g., species differences in the depth of rooting as estimated from foliar cation concentrations; Table 1) (see below) contribute to differences in injury expression in the field. Rooting depth may be particularly important because field data show a dramatic attenuation in the extent and duration of freezing in soils from 7.5 to 15 cm (Hennon et al., 2010) and from 10 to 20 cm (D'Amore et al., 2010) when a snowpack is present.

Although species differed in the extent and variability of root cold hardiness attained, soil temperatures at both depths (Fig. 3) never dropped below the cold tolerance thresholds estimated for any species (Fig. 2) because snow was present during these periods. Therefore, it seems likely that roots from all species escaped freezing injury during the months of this assessment.

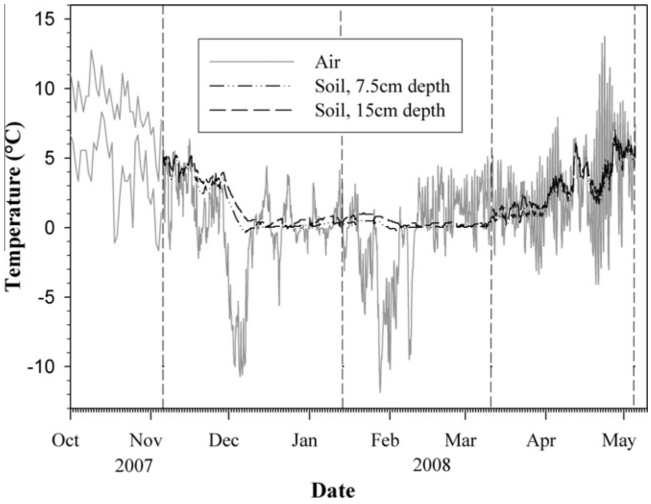

Fig. 3. Hourly air temperatures and soil temperatures at two depths for the Ward Lake sample site near Ketchikan, Alaska. Sample dates are indicated with dashed vertical lines. Air temperatures before the first sample dates are maximum and minimum temperatures recorded at the Ketchikan Airport. Other data were recorded at the sample site and represent the means of 4 devices deployed along the sampling transect on the first sample date.

3.2. Membrane electrolyte leakage

For all sample times combined, REL levels of roots (baseline electrolyte leakage from roots prior to experimental freezing tests) were highest for yellow-cedar, next highest for western redcedar, and lowest for the hemlocks and Sitka spruce, which were

Table 1
Mean foliar calcium (Ca) and aluminum (Al) concentrations and molar Ca:Al ratios (±SE) by species for yellow-cedar (YC), redcedar (RC), mountain hemlock (MH), western hemlock (WH) and Sitka spruce (SS) trees growing together in Ketchikan, AK. Foliage was collected in January 2008. Treatment means with different letters are significantly different ($P \leqslant 0.05$) based on the following orthogonal contrasts: (1) YC and RC versus MH, WH, and SS, (2) YC versus RC, (3) MH and WH versus SS, and (4) MH versus WH.

	Species mean ± SE					Significance of contrast (*P* value)			
Foliar cation	YC	RC	MH	WH	SS	YC and RC vs. MH, WH and SS	YC vs. RC	MH and WH vs. SS	MH vs. WH
Ca (mg/kg)	10570.8 ± 1028.9	7110.6 ± 339.4	1874.6 ± 190.2	2449.6 ± 267.5	2170.9 ± 410.8	<0.001	0.010	0.961	0.121
Al (mg/kg)	22.3 ± 4.2	24.1 ± 3.6	512.6 ± 160.6	182.8 ± 17.2	76.0 ± 53.3	<0.001	0.753	<0.001	0.106
Ca:Al	333.9 ± 38.0	213.6 ± 29.5	3.5 ± 0.9	8.7 ± 0.6	54.1 ± 19.1	<0.001	0.031	0.001	0.090

statistically indistinguishable from one another (Fig. 4). For all species combined, roots had the lowest REL levels in November and the highest in May, with January and March measurements being intermediate (Fig. 4). There was a significant month × species interaction for root REL ($P < 0.01$), which was driven by the comparatively minor changes in REL for yellow-cedar and to a lesser extent western redcedar, relative to the temporal variations for the other species (Fig. 5). Yellow-cedar and redcedar roots had higher REL values on all dates than the other species (Fig. 5). High REL measurements can reflect various differences in physiology, notably including (1) root injury (e.g., freezing damage; Schaberg et al., 2008), and (2) greater electrolyte loss associated with the growth and activity of non-suberized roots (McKay, 1998). Because soil temperatures (Fig. 3) never fell below estimated cold tolerance levels (Fig. 2) it seems likely that elevated REL levels were not the result of freezing injury, but represented a greater potential for root activity.

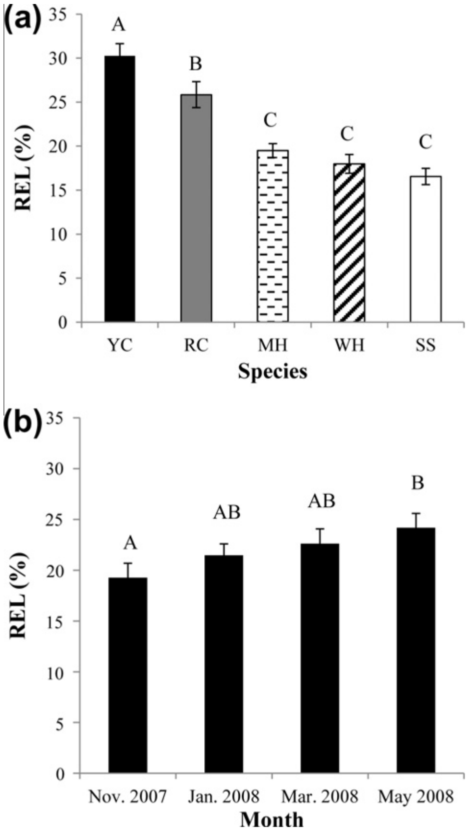

Fig. 4. (a) Differences in overall mean (±SE) relative electrolyte leakage (REL) before freezing treatments of fine roots of yellow-cedar (YC), western redcedar (RC), mountain hemlock (MH), western hemlock (WH) and Sitka spruce (SS) growing in Ketchikan, Alaska during the 2007–2008 cold season. Species means with different letters are significantly different ($P \leqslant 0.05$) based on the following orthogonal contrasts: (1) YC and RC versus MH, WH, and SS, (2) YC versus RC, (3) MH and WH versus SS, and (4) MH versus WH. (b) Differences in mean (±SE) relative electrolyte leakage (REL) before freezing treatments of fine roots for all species for each sample month. Monthly means with different letters are significantly different ($P \leqslant 0.05$) based on Tukey HSD test.

Differences in REL for yellow-cedar and western redcedar were significant in November and March – times of transition in temperature and likely physiological activity. During these transitions, yellow-cedar showed greater membrane leakiness than western redcedar, suggesting that yellow-cedar roots were more physiologically active than co-occurring redcedar. A propensity for greater root activity when other species are less active is consistent with the proposition that yellow-cedar may be physiologically more opportunistic – exhibiting elevated activity throughout the year when environmental conditions are favorable (Puttonen and Arnott, 1994; Grossnickle and Russell, 2006). Other studies have shown that yellow-cedar can photosynthesize at low (even sub-freezing) temperatures (Grossnickle and Russell, 2006), and have significant root growth in cold (but not frozen) soils (Arnott et al., 1993). The consistently limited cold tolerance of yellow-cedar roots across all samples dates (Fig. 2) supports this proposed capacity for opportunistic activity because tissues compromise some capacity for metabolic productivity in order to achieve greater cold tolerance (Schaberg, 2000). The capability for precocious root activity is also consistent with the possibility that yellow-cedar takes up N as NO_3^- in the spring when other species exhibit less uptake capacity (D'Amore et al., 2009). The compromise between cold tolerance and activity is likely not a risky tradeoff for yellow-cedar trees when roots are protected from low air temperatures by a consistent insulative snowpack. However, as climatic warming reduces the average depth and duration of snowpack, what may have been an adaptive benefit allowing yellow-cedar roots to access a little-tapped source of N, may become a physiological liability as the risks of freezing injury outweigh potential nutritional advantages (D'Amore et al., 2009).

3.3. Foliar cations as indicators of rooting depth

Along with differences in cold tolerance, yellow-cedar and western redcedar foliage had significantly higher Ca, lower Al, and higher molar ratios of Ca:Al than foliage from the other species examined (Table 1). Yellow-cedar foliage had higher concentrations of Ca and higher ratios of Ca:Al than did western redcedar foliage. The species exhibited an over fivefold range in foliar Ca concentrations and an over 20-fold range in foliar Al concentrations, with yellow-cedar and mountain hemlock representing the extremes in both ranges.

Soil cation analysis indicated that the surface horizon had over two times the extractable Ca and about 44% the extractable Al than the subsurface horizon (Table 2). As a consequence, the surface horizon had a nearly threefold higher molar Ca:Al ratio than the subsurface horizon (Table 2). Concentrations of Ca are often greatest in the upper organic horizons of forest soils as a result of the biocycling of base cations (e.g., Likens et al., 1998), especially in cedar forests because of the consistently high concentrations of

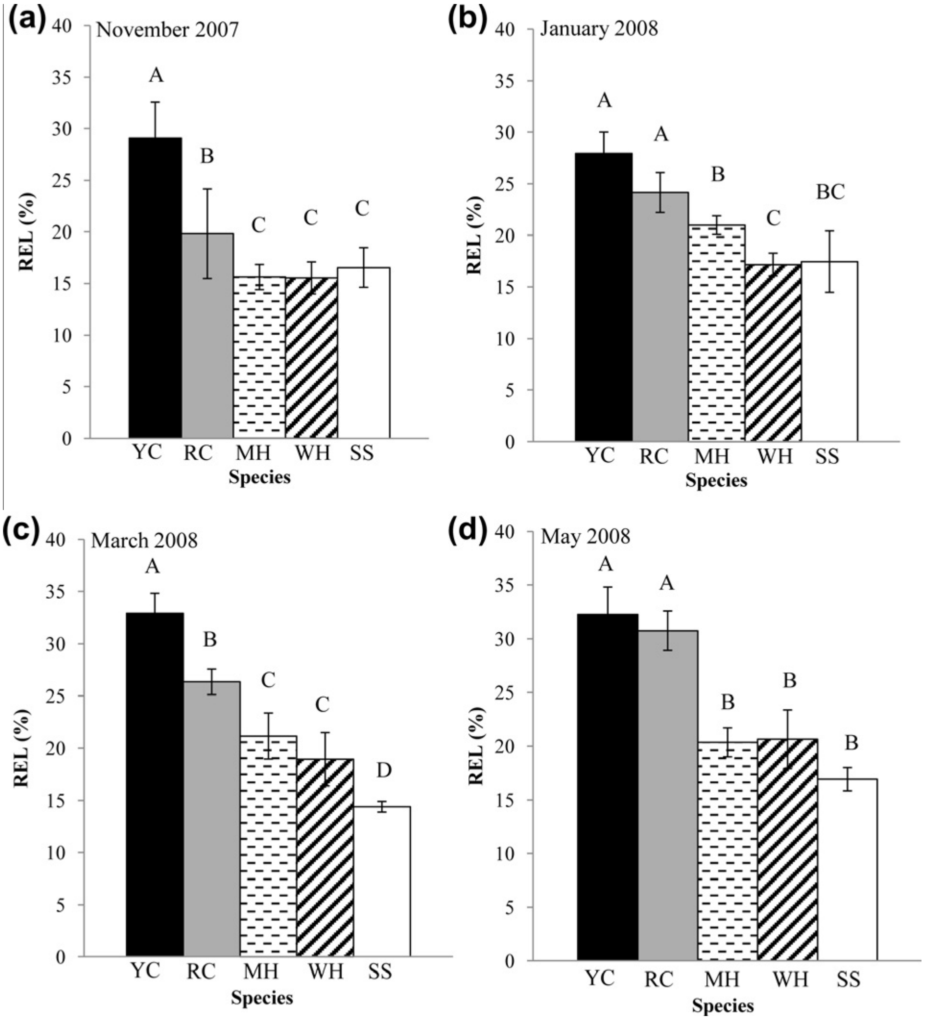

Fig. 5. Differences in mean (±SE) relative electrolyte leakage (REL) before freezing treatments of fine roots of yellow-cedar (YC), western redcedar (RC), mountain hemlock (MH), western hemlock (WH) and Sitka spruce (SS) growing together in Ketchikan, Alaska and assessed on four dates: (a) November 2007, (b) January 2008, (c) March 2008, and (d) May 2008. Per sampling date, treatment means with different letters are significantly different ($P \leqslant 0.05$) based on the following orthogonal contrasts: (1) YC and RC versus MH, WH, and SS, (2) YC versus RC, and (3) MH and WH versus SS.

Table 2
Mean calcium (Ca) and aluminum (Al) concentrations adjusted for bulk density (mg/cm^3) and molar Ca:Al ratios (±SE) for soils surrounding sample trees in Ketchikan, AK. Soil samples were collected from organic (0–5 cm deep) and mineral (15–20 cm deep) horizons in November 2010. Means are considered statistically different between horizons at $P < 0.05$ based on ANOVA analyses.

Soil cation	Sampling depth		*P*-value
	Surface horizon	Subsurface horizon	
Ca (mg/cm^3)	0.602 ± 0.097	0.253 ± 0.081	0.009
Al (mg/cm^3)	0.026 ± 0.004	0.047 ± 0.007	0.014
Ca:Al	29.88 ± 8.71	10.05 ± 4.35	0.001

Ca in cedar foliage (D'Amore et al., 2009). Calcium also accumulates in the forest floor due to the formation of Ca-organic matter precipitates, such as Ca-humates (Zech et al., 1990). For these and other reasons, Ca is concentrated in surface horizons of soils in southeast AK (Heilman, 1968; D'Amore and Hennon, 2006) and British Columbia (Kranabetter and Banner, 2000). Therefore, the high extractable values of Ca in surface soils are reasonable given the behavior of Ca and our extraction method (1 M KCl). The values for Al are somewhat more complicated due to the complex, multiphase components of Al that exist in soils (Bertsch and Bloom, 1996). The extraction method we used provides a soil- and method-specific quantification of Al, but it does not allow for the explicit quantification of the interaction among all Al components in the soils. Nonetheless, our results provide an indicator of the relationship between extractable Al and Ca in the soils at the two sample depths. From this relationship we conclude that the higher extractable Al in sub-surface soils than surface soils is consistent with the influence of pH on Al solubility and retention in organic soils (Walker et al., 1990) and Al migration downward in soils due to the metal-fulvate theory of podzolization (Duchaufour, 1982; McKeague et al., 1983). The concentration of extractable Al in surface soils is typically low because plants have various adaptations to limit the bioavailability and transport of Al to foliage (Kochian et al., 2005; Poschenrieder et al., 2008) that then abscises and decomposes to help generate this horizon. In contrast, deeper horizons have a pool of Al species that are associated with mineral and organic material due to the podzolization process (Dahlgren and Ugolini, 1989).

As a result of various biogeochemical processes, both the surface and subsurface horizons have characteristic patterns of Ca and Al availability that can be used as indicators of the relative exploitation of these horizons by roots via transpirational uptake to foliage. In particular, higher concentrations of Ca in foliage should reflect greater rooting in the upper organic horizon, higher foliar concentrations of Al should reflect greater rooting in the lower horizon, and foliar Ca:Al should represent the relative proportion of roots in the surface versus subsurface horizons. Consistent with this, across all trees and associated soil samples, foliar Ca concentrations (mg/kg) had a stronger linear association with surface horizon Ca concentrations ($P < 0.001$, $R^2 = 0.84$) than with subsurface Ca availability ($P < 0.02$, $R^2 = 0.36$), both expressed as mg/g. Similarly, foliar Al concentrations were not linearly related to Al concentrations of the surface organic horizon ($P = 0.29$, $R^2 = 0.09$), but were related to the availability of Al in the subsurface horizon ($P = 0.047$, $R^2 = 0.29$).

There are various physiological processes in addition to rooting depth that influence Ca and Al uptake and accumulation in foliage. For example, a prominent factor that could influence foliar Ca incorporation is a variation in annual transpirational uptake among species. Several studies have shown that spruce species experience greater cumulative transpirational losses when added across the seasons compared to various conifer and hardwood species (see review by Pallerdy et al., 1995). Even with a predicted greater transpiration and associated Ca uptake for spruce, we found Sitka spruce to have foliar Ca concentrations indistinguishable from the hemlocks and almost five times lower than yellow-cedar (Table 1). Furthermore, numerous mechanisms limit Al uptake and movement to leaves (e.g., Marschner, 2002), which would reduce the congruence of soil and foliar Al concentrations relative to Ca. Despite this, our data showed a general increase in foliar Al with increasing Al in the subsurface horizon, suggesting that what little Al does accumulate in foliage likely originates from deeper soil horizons. Although variations in Al exclusion, transpiration and other physiological processes likely influenced the species differences in foliar Ca and Al levels that we found, the significant linear relationships between soil and foliar cation concentrations suggests that differential root access to soil pools is also an important contributor to foliar Ca and Al accumulations.

Considering the distribution of cations among soil horizons, the higher concentration of Ca and the higher Ca:Al ratio in the foliage of yellow-cedar suggest that its roots disproportionally occupy (and take up cations from) higher soil horizons than the other species. This finding is consistent with favorable fine rooting of subalpine trees in soil horizons that had lower Al:Ca molar ratios (Dahlgren et al., 1991). Furthermore, foliar nutrients suggest that redcedar occupies an intermediate rooting position between shallow rooted yellow-cedar and more deeply rooted hemlock and spruce. There is evidence that western redcedar is not uniformly shallow rooted. Bennett et al. (2002) reported that western redcedar fine root biomass was located in both surface soils and deeper mineral horizons in mixed conifer stands, and Wang et al. (2002) found that redcedar root mass was concentrated in the upper most mineral soil horizon in pure cedar stands. Despite important differences in the cold tolerance and depth of roots that seem to place yellow-cedar at a uniquely elevated risk of injury, the cold tolerance and rooting depths of yellow-cedar and western redcedar are similar enough that one might expect western redcedar to show some, though more limited, vulnerability to root freezing injury. Indeed, it has been proposed that the dead tops (i.e., spike-tops) seen in some mature redcedars might be an indicator of some limited root freezing injury rather than a symptom of Ca deficiency, as once thought (D'Amore et al., 2009). The possible connections between soil freezing and the spike-tops of western redcedar have yet to be evaluated. This has important management implications because one possible strategy is to favor redcedar in forests impacted by yellow-cedar decline where it can contribute somewhat similar cultural, economic, and ecological functions as yellow-cedar.

The roots of many species occupy portions of the upper organic soil horizons that contain high concentrations of essential nutrients such as Ca. Accordingly, when these shallow regions of soil freeze, we propose that the superficial roots of numerous species are likely damaged. However, for species with some abundance of deep roots, losses of surface roots to freezing injury are unlikely to substantially reduce water uptake and induce foliar desiccation in the spring – especially in the wet, cool climates of the North Pacific temperate rainforest where vernal transpirational demand is limited. However, by concentrating roots in upper soil horizons, yellow-cedar would have a greater risk of experiencing extensive root freezing injury because when no snow is present soil freezing is common and reaches lethal temperatures at soil depths of 7.5 cm, but is rare and more muted at 15 cm (D'Amore and Hennon, 2006; Hennon et al., 2010).

4. Conclusions

Our data from one study site indicate that yellow-cedar roots are less cold tolerant than the roots of several sympatric conifers, and are slightly less cold tolerant than the roots of western redcedar in fall and mid winter. Yellow-cedar roots were also fully dehardened in March, whereas the roots of other species continued to deharden into May. Furthermore, we infer from foliar Ca and Al concentrations that yellow-cedar has more fine roots concentrated in surface soil horizons than other associated conifers – including western redcedar. This combination of limited cold tolerance and enhanced superficial rooting could be of adaptive benefit by allowing for cold season nitrate uptake (D'Amore et al., 2009), but it would also put yellow-cedar at elevated risk of broad-scale root freezing injury when snowpack levels were low and chances for soil freezing increased. Overall, our data indicate that both limited root cold tolerance and shallow rooting likely contribute to the unique sensitivity of yellow-cedar to freezing injury and decline relative to sympatric conifers.

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